

# The diversity of soil culturable fungi in the three alpine shrub grasslands of Eastern Qilian Mountains

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**Abstract** To understand the diversity of culturable fungi in soil at alpine sites, *Rhododendron fruticosum* shrubland, *Salix cupularis fruticosum* shrubland, and *Dasiphora fruticosum* shrubland of the Eastern Qilian Mountains were selected to investigate. Three methods, including traditional culturing, rDNA internal transcribed spacer (ITS) sequence analysis, and economical efficiency analysis, were carried out to estimate the diversity of soil culturable fungi of these three alpine shrublands. A total of 35 strains of culturable fungi were cultured by dilution plate technique and were analyzed by rDNA ITS sequence. The diversity indices such as species abundance (*S*), Shannon–Wiener index (*H*), Simpson dominance index (*D*), and Pielou evenness index (*J*) of *Rhododendron fruticosum* shrubland, *Salix cupularis fruticosum* shrubland, and *Dasiphora fruticosum* shrubland were ranged between 16 and 17, 2.66–2.71, 0.92, 0.95–0.97 respectively. The results showed that the diversity of soil fungi were abundant in these three types of alpine shrub grasslands, while further study should be done to explore their potential value.

**Keywords** alpine shrub grasslands, culturable fungi, rDNA ITS sequence analysis, diversity

## 1 Introduction

Fungi play an important role in the soil ecosystem. As only a small fraction of the fungi present in soil can be cultured, and conventional microbiological techniques yield only limited information on the composition and dynamics of fungal communities in soil. Soil is the most diverse of

terrestrial habitats, and the soil microbial community plays a fundamental role in decomposition, nutrient cycling, and energy flow (Wardle and Giller, 1996). Soils with high biodiversity appear to be more resistant to stress (Griffiths et al., 2000), and low diversity may be associated with impaired ecosystem function (Tilman et al., 1997). In details, fungi are important as decomposers of organic matter and comprise a major proportion of soil microbial biomass. In grasslands, 78%–90% of the total decomposer biomass may be fungal (Kjøller and Struwe, 1982). Fungi also function as plant and insect pathogens, predators, and mutualisms. Mycorrhizal plant symbioses are ubiquitous and often essential for plant survival in dry or nutrient-poor conditions (Allen and Allen, 1992). Changes in fungal diversity associated with agricultural management may, therefore, have important implications for soil fertility, stability, plant establishment, and yield. Although it is known that fungal communities are affected by agricultural practices (Bardgett et al., 1993), the effects of agricultural intensification on fungal biodiversity remain unclear.

There are a number of difficulties involved in studying soil fungal biodiversity. Conventional microbiological culture techniques are thought to detect < 1% of bacteria present in the soil because of the selectivity of growth media and conditions (Borneman et al., 1996), and it is likely that fungal presence is similarly underestimated (Hawksworth, 2001). Several fungal groups, such as arbuscular mycorrhizas (Gams, 1992) and some basidiomycetes, are difficult or impossible to culture, and isolation onto agar media favors fast-growing, heavily sporulating species (Frankland et al., 1990). Thus isolation techniques do not provide an accurate picture of the in situ diversity of the active members of fungal communities. Fungi fulfil a range of important ecological functions, yet current understanding of fungal diversity in soil at an alpine site in the Eastern Qilian Mountains is limited. The objective of

this study is to evaluate the diversity of fungi at three alpine shrublands and illustrate their relative abundance in soils at alpine sites in the Eastern Qilian Mountains.

## 2 Materials and methods

### 2.1 Study site

The site of this study is located on the Jinqiang River of Tianzhu County (37°11'–37°18' N, 102°23'–102°78' E, elevation 2700–3300 m), located in the Eastern Qilian Mountains of Gansu Province. The mean annual temperature was  $-0.1^{\circ}\text{C}$ , with minimum temperature  $18.3^{\circ}\text{C}$  in January and maximum temperature  $12.7^{\circ}\text{C}$  in July 2009. The annual precipitation in the region is 416 mm. The cold season lasts seven months from the end of October until April of the next year while the growing season only lasts four months. The soil of the region is lean with a 16.09%–36.25% water content and 6.47–7.71 pH value. The basic status of the sampling sites is described in detail in Table 1. Five soil samples were taken randomly at each sampling region in July. Soil cubes (20 cm  $\times$  20 cm  $\times$  20 cm) were cut with a sterile sharp knife from the upper layer at each sampling site. All samples were placed in sterile bags and stored at  $4^{\circ}\text{C}$  in the microbiological laboratory. All samples were processed within 24 h.

### 2.2 Isolates and morphology

A traditional culturing method was used to isolate the culturable fungi in the soil samples. 10-fold dilutions

obtained from a suspension of each sample were used to inoculate on a non-selective but fungus-specific Sabouraud solid culture medium. For each composite sample, three repetitions were made and five Petri dishes were inoculated for each dilution. The isolates were grown on potato dextrose agar (PDA), potato sugar agar (PSA), maize powder media and rose bengal medium plates in darkness at  $25^{\circ}\text{C}$  until they completely covered the medium surface. Colony forming units were counted after culture in five days and the fungal strains were morphologically distinguished and their frequencies in each sample were used to compare the mycoflora at the qualitative level.

### 2.3 Isolates and morphology

A DNA extraction protocol optimized for fungal strains was developed. The mycelium was scraped off and collected in a 2 mL Eppendorf tube with 50  $\mu\text{L}$  of autoclaved micro glass micro bead (230–320  $\mu\text{m}$  diam.). The tubes were then placed in liquid nitrogen for 5 min and transferred to ice. To separate organic and aqueous phases, 250  $\mu\text{L}$  of phenol and 250  $\mu\text{L}$  of chloroform were added, together with 500  $\mu\text{L}$  of lysis buffer (100 mM NaCl, 10 mM Tris-HCl pH 8.0, 1 mM EDTA, 2% Triton X-100, 1% SDS). Tubes were vortexed for 20 min and then centrifuged (19000  $\times$  g,  $4^{\circ}\text{C}$ , 25 min). The aqueous phase was transferred to a new 1.5 mL tube and the nucleic acids precipitated with an equal volume of cold absolute isopropanol. The tubes were centrifuged again (19000  $\times$  g,  $4^{\circ}\text{C}$ , 10 min), the supernatants discarded and the pellets washed with 1 mL of cold 70% ethanol. After a further centrifugation (19000  $\times$  g,  $4^{\circ}\text{C}$ , 5 minutes), the super-

**Table 1** Basic status of the sampling sites

Shrub grassland types	Altitude/m	Geographic coordinate	Soil types	pH value	Use types	Vegetation condition
<i>Rhododendron fruticosum</i> shrubland	3220	37.16°E 102.77°N	Alpine shrubs meadow soil	7.71 $\pm$ 0.03	Winter pasture	<i>Rhododendron anthopogonoides</i> , <i>Rhododendron thymifolium</i> , <i>Rhododendron qinghaiense</i> and <i>Rhododendron capitatum</i> are the dominant species in the site, the coverage of shrubs are around 54%.
<i>Salix cupularis fruticosum</i> shrubland	3130	37.17°E 102.76°N	Alpine shrubs meadow soil	6.47 $\pm$ 0.04	Winter pasture	<i>Salix taiwanalpina</i> is the absolute dominant species, and <i>Potentilla fruticosa</i> is the companion species, the coverage of shrubs are around 78%.
<i>Dasiphora fruticosum</i> shrubland	3020	37.18°E 102.77°N	Subalpine shrubs meadow soil	7.45 $\pm$ 0.02	Winter pasture	<i>Potentilla fruticosa</i> is the absolute dominant species and <i>Salix taiwanalpina</i> is the companion species in the site, coverage of shrubs are around 75%.

natants were discarded and the pellets dried at RT with the tubes open in an inverted position. RNA was digested by incubating the pellets with 50  $\mu$ L of TE (10 mM Tris, 1 mM EDTA) + RNase A (Sigma®) (50  $\mu$ g  $\cdot$  mL<sup>-1</sup>) at 55°C for 15 min.

#### 2.4 rDNA internal transcribed spacer (ITS) gene amplification, sequencing and phylogenetic tree analysis

The primers set ITS1 (5'-TCC GTA GGT GAA CCT GCG C-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') (White et al., 1990) was used to amplify the rDNA ITS region. The PCR reaction mixture consisted of 50–100 ng of genomic DNA, 15 pmol of each primer, 200  $\mu$ M of each dNTP, 3 mM MgCl<sub>2</sub>, 1% DMSO, 1 U Taq DNA polymerase (recombinant) (Fermentas Life Sciences, Ontario, Canada) and was made up to a total volume of 50  $\mu$ L. The cycling conditions were: 5 min at 95°C, followed by 40 cycles of 1 min at 94°C, 30 s at 50°C, and 1 min at 72°C, and a final step of 10 min at 72°C. The amplicons were purified with illustra™ GFX™ PCR DNA and Gel Band Purification Kit (GE Healthcare Life Sciences) according to the manufacturer's instructions. The ITS region was sequenced in both directions by STAB VIDA, Lda. using primer ITS1 and ITS4 (White et al., 1990). Sequences were edited with BioEdit v.7.0.9.0 (Hall, 1999) and aligned with ClustalX (1.83) (Thompson et al., 1997). Additional sequences were obtained from GenBank using BLAST (Basic Local Alignment Search Tool) (Altschul et al., 1990) and included in the alignment. Terminal regions, with missing data in some of the isolates, were excluded from the analysis. All molecular characters were unordered and given equal weight during the analysis. Phylogenetic trees were inferred in PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford, 2002) by Neighbor-Joining (NJ) using Kimura 2-parameter distance and maximum parsimony (MP) using the heuristic search option with random addition of sequences (1000 replications), tree bisection-reconnection (TBR) and MULTREES options ON. Gaps were regarded as a fifth character in MP. Bootstrap support values with 1000 replications (Felsenstein, 1985) were calculated for tree branches in both phylogenetic methods. MP bootstrap analysis was done with the MULTREES option off and 10 random sequence additions in each of 1000 pseudoreplicates. Sequences obtained from GenBank are listed by their accession numbers and taxon names in the tree, while newly generated sequences are listed by their isolate number. Newly generated sequences have been deposited in GenBank and the alignment and phylogeny in TreeBASE (Matrix M4195; Study S2209).

#### 2.5 Diversity of soil fungi

According to the identified fungi, the characteristics of each measure index and type of sampling data, the fungi

diversity were analyzed by ecology-evaluating methods.

The species richness using Eq. (1):

$$S = N, \quad (1)$$

where,  $N$  is the total number of genus.

The Shannon–Wiener diversity index ( $H$ ) (Atlas and Bartha, 1998) was used to estimate fungal diversity based on the number of identification genus using Eq. (2):

$$H = - \sum_{i=1}^s Pi \ln Pi, \quad (2)$$

where  $Pi = n_i/N$ ,  $N = \sum_{i=1}^s Ni$ ,  $n$  is the total number of the genus, and  $N$  is the total number of genus in the sample.

Pielou ( $J$ ) was calculated according to Eq. (3):

$$J = \sum_{i=1}^s Pi \ln Pi / \ln S, \quad (3)$$

where  $Pi = n_i/N$ ,  $N = \sum_{i=1}^s Ni$ ,  $n$  is the total number of the genus, and  $N$  is the total number of genus in the sample.

Simpson ( $D$ ) was calculated according to Eq. (4):

$$D = 1 - \sum_{i=1}^s Pi^2 \quad (4)$$

where  $Pi = n_i/N$ ,  $N = \sum_{i=1}^s Ni$ ,  $n$  is the total number of the genus, and  $N$  is the total number of genus in the sample.

## 3 Results

### 3.1 Quantity and abundance of fungi

The quantity and percentages of fungi are shown in Table 2. The most dominant genera isolated from soil samples was *Doratomyces*, which accounted for 28.32%, 12.12%, and 12.25% in *Rhododendron fruticosum* shrubland, *Salix cupularis fruticosum* shrubland and *Dasiphora fruticosum* shrubland, respectively. *Bionectria* was the commonly isolated genera in the *Rhododendron fruticosum* shrubland and *Salix cupularis fruticosum* shrubland, which was the second large group. In the *Dasiphora fruticosum* shrub, the genera of *Mortierella* and *Scopulariopsis* were the second largest group. It should also be noted that the only fungi detected by culturing methods was the genus of *Verticillium* in the *Rhododendron fruticosum* shrubland and the genus of *Penicillium* in the *Dasiphora fruticosum* shrub. The genera of *Aspergillus* and *Geomyces* were only isolated in the *Salix cupularis fruticosum* shrubland. Although the culture media was carefully chosen to be as

**Table 2** The quantity and percentages of fungi

Taxonomic status	<i>Rhododendron fruticososa</i> shrubland		<i>Salix cupularis fruticosa</i> shrubland		<i>Dasiphora fruticosa</i> shrubland	
	Quantity/( $\times 10^4$ cfu·g <sup>-1</sup> )	Percentage/%	Quantity/( $\times 10^4$ cfu·g <sup>-1</sup> )	Percentage/%	Quantity/( $\times 10^4$ cfu·g <sup>-1</sup> )	Percentage/%
<i>Mortierella</i>	0.39	7.59	0.29	5.79	0.49	12
<i>Doratomyces</i>	28.13	28.32	14.35	12.12	11.08	12.25
<i>Leptosphaeria</i>	0.39	7.59	0.29	5.79	0.11	2.66
<i>Cladosporium</i>	0.13	2.53	0.29	5.79	0.16	4
<i>Verticillium</i>	0.19	3.79	0	0	0	0
<i>Mucor</i>	0.45	8.86	0.36	7.24	0.27	6.66
<i>Tolypocladium</i>	0.13	2.53	0	0	0.16	4
<i>Beauveria</i>	0.13	2.53	0.14	2.89	0	0
<i>Hypocrea</i>	0	0	0.07	1.44	0.11	2.66
<i>Truncatella</i>	0.32	6.32	0.22	4.34	0.22	5.33
<i>Cordyceps</i>	0.06	1.26	0	0	0.11	2.66
<i>Trichoderma</i>	0.26	5.06	0.44	8.69	0.16	4
<i>Nectria</i>	0	0	0	0	0.11	2.66
<i>Didymella</i>	0	0	0	0	0.16	4
<i>Bionectria</i>	0.71	13.92	0.51	10.14	0.49	12
<i>Alternaria</i>	0.39	7.59	0.22	4.34	0.11	2.66
<i>Penicillium</i>	0	0	0	0	0.16	4
<i>Geomyces</i>	0	0	0.07	1.44	0	0
<i>Fusarium</i>	0.32	6.32	0.44	8.69	0.11	2.66
<i>Scopulariopsis</i>	0.45	8.86	0.44	8.69	0.44	10.66
<i>Aspergillus</i>	0	0	0.07	1.44	0	0
<i>Phaeosphaeria</i>	0.19	3.79	0.29	5.79	0	0
Other genera	0.52	10.12	0.66	13.04	0.49	12

non-selective as possible, some species were not isolated probably because the culturing conditions and the culture medium were not appropriate for their growth. Some species were isolated in one sample site while another was not, probably because the diversity of soil fungi was different among sample sites. More research needs to be done on the culture conditions of the soil fungi and their phylogenetic relationships.

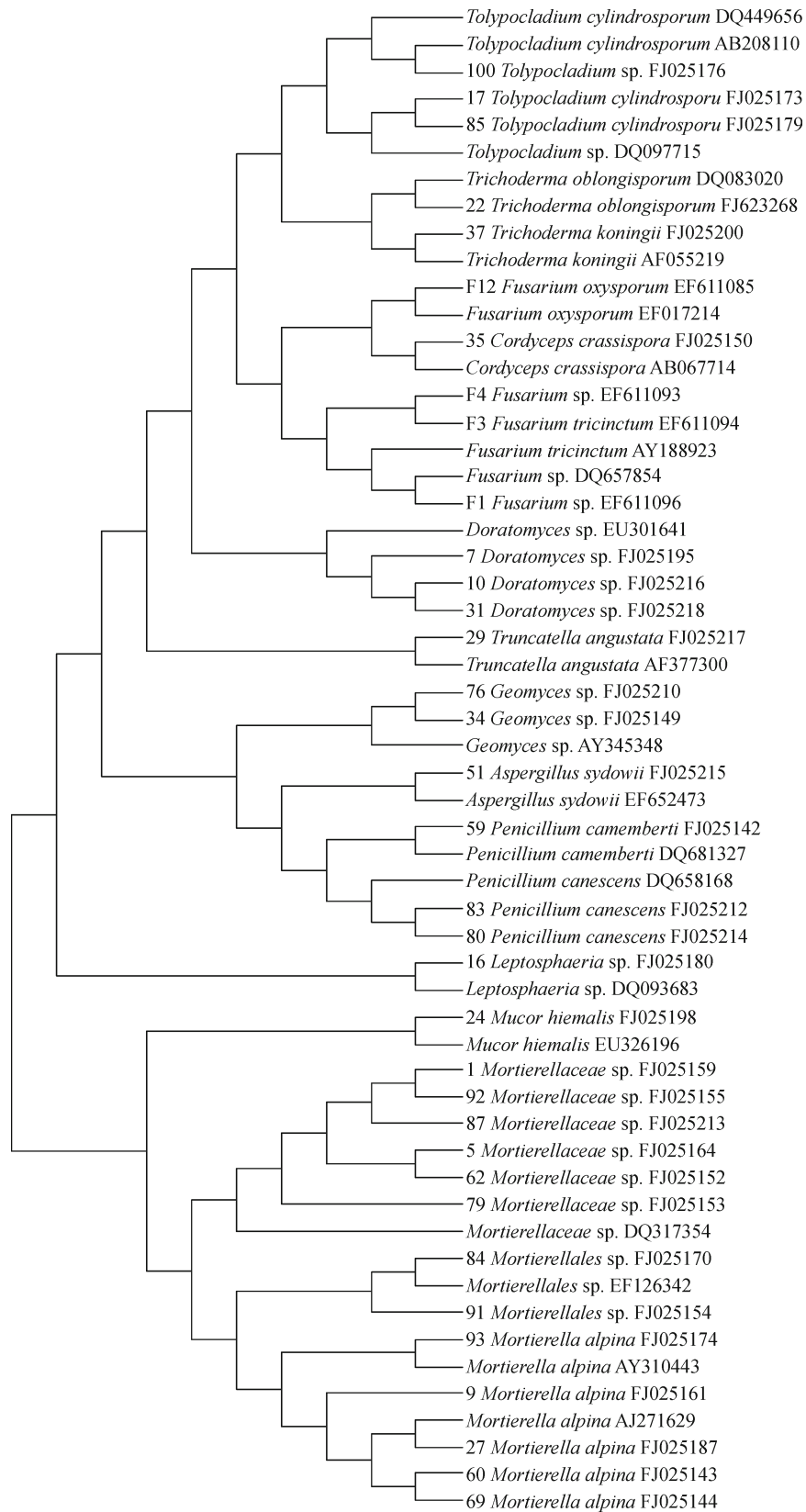
### 3.2 rDNA ITS gene amplification, sequencing and phylogenetic tree analysis

The study shows rDNA ITS sequence analysis and traditional culturing used together to provide more accurate information on fungal diversity in soil at alpine sites. A total of 96 strains were inoculated in suitable conditions but only 35 strains were cultured and subcultured successfully. Based on the cultural characteristics and morphological types, a total of 35 out of 96 strains were analyzed by traditional culturing methods and molecular methods. The sequences of the ITS1-5.8S-ITS2 region were obtained from 35 strains and the fragment length of each sequence was from 499 to 653 bp. The

sequences were compared with the data available in GenBank. The rDNA ITS region of the selected isolates was sequenced and BLAST searches were done to select closely related sequences. The sequences giving the best matches in each case were aligned manually and the percentage of similarity was calculated based on the number of conserved positions. BLAST identifications based on the sequences revealed a total of 22 different genera. All relationships were confirmed after the phylogenetic analysis (Fig. 1). A total of 35 out of 96 typical strains identified have 98% identity on BLAST matches, indicating that their phylogenetic relationships are clear and these species possibly belong to those previously reported (Table 3). The molecular data from these 35 isolates agreed with the morphology at the genus level. We observed a reasonable agreement between the sequence data and the morphological classification, yielding positive results from each method.

### 3.3 Diversity of soil fungi

The fungi diversity was analyzed by ecology-evaluating methods. The range of species abundance ( $S$ ),  $H$ ,  $D$  and  $J$



**Fig. 1** Phylogenetic analysis of the alpine grasslands of soil fungi diversity based on rDNA ITS sequences

**Table 3** Phylogenetic relation to nearest neighbors of strains isolated from alpine shrublands soil

Number of strain (Accession number)	Fragment length/bp	Reference taxa/(Accession number)	Similarity/%
1 (FJ025159)	634	<i>Mortierellaceae</i> sp. (DQ317354)	99
7 (FJ025195)	581	<i>Doratomyces</i> sp. (EU301641)	98
16 (FJ025180)	556	<i>Leptosphaeria</i> sp.(DQ093683)	98
17 (FJ025173)	639	<i>Tolypocladium cylindrosporium</i> (AB208110)	99
22 (FJ623268)	610	<i>Trichoderma oblongisporum</i> (DQ083020)	99
27 (FJ025187)	650	<i>Mortierella alpina</i> (AJ271629)	98
34 (FJ025149)	638	<i>Geomyces</i> sp. (AY345348)	99
59 (FJ025142)	651	<i>Penicillium camemberti</i> (DQ681327)	99
79 (FJ025153)	634	<i>Mortierellaceae</i> sp. (DQ317354)	99
85 (FJ025179)	499	<i>Tolypocladium cylindrosporium</i> (DQ449656)	99
93 (FJ025174)	631	<i>Mortierella alpina</i> (AY310443)	99
F1 (EF611096)	534	<i>Fusarium</i> sp. (DQ657854)	100
F4 (EF611093)	537	<i>Fusarium</i> sp. (DQ657854)	100
9 (FJ025161)	647	<i>Mortierella alpina</i> (AJ271629)	98
10 (FJ025216)	579	<i>Doratomyces</i> sp. (EU301641)	97
29 (FJ025217)	564	<i>Truncatella angustata</i> (AF377300)	97
37 (FJ025200)	586	<i>Trichoderma koningii</i> (AF055219)	99
51 (FJ025215)	544	<i>Aspergillus sydowii</i> (EF652473)	99
62 (FJ025152)	640	<i>Mortierellaceae</i> sp. (DQ317354)	99
69 (FJ025144)	653	<i>Mortierella alpina</i> (AJ271629)	98
83 (FJ025212)	554	<i>Penicillium canescens</i> (DQ658168)	99
87 (FJ025213)	630	<i>Mortierellaceae</i> sp. (DQ317354)	98
91 (FJ025154)	640	<i>Mortierellales</i> sp. (EF126342)	99
5 (FJ025164)	634	<i>Mortierellaceae</i> sp. DQ317354	99
24 (FJ025198)	622	<i>Mucor hiemalis</i> EU326196	99
31 (FJ025218)	579	<i>Doratomyces</i> sp. EU301641	98
35 (FJ025150)	634	<i>Cordyceps crassispora</i> AB067714	99
60 (FJ025143)	652	<i>Mortierella alpina</i> AJ271629	97
76 (FJ025210)	531	<i>Geomyces</i> sp. AY345348	99
80 (FJ025214)	552	<i>Penicillium canescens</i> DQ658168	99
84 (FJ025170)	645	<i>Mortierellales</i> sp. EF126342	98
92 (FJ025155)	633	<i>Mortierellaceae</i> sp. DQ317354	99
100 (FJ025176)	646	<i>Tolypocladium</i> sp. DQ097715	99
F3 (EF611094)	540	<i>Fusarium tricinctum</i> AY188923	100
F12 (EF611085)	519	<i>Fusarium oxysporum</i> EF017214	99

Note: Representative isolates were subsequently selected for a molecular phylogeny based on the rDNA ITS region (ITS1–5.8S–ITS2)

in *Rhododendron fruticosum* shrubland, *Salix cupularis fruticosa* shrubland and *Dasiphora fruticosa* shrubland was 16–17, 2.66–2.71, 0.92, 0.95–0.96, respectively (Table 4). *H* changed from 2.66 to 2.71, which means that three types of alpine shrub grasslands were higher in

the diversity of soil fungi. *J* was close to 1, which illustrates that the soil fungi was widely distributed in the three alpine shrub grasslands. The results show that the diversity of soil fungi was abundant in three types of alpine grasslands, which warrants further study.

**Table 4** Soil fungi diversity of whole community and different samples

Diversity index	<i>Rhododendron fruticosum</i> shrubland	<i>Salix cupularis fruticosum</i> shrubland	<i>Dasiphora fruticosum</i> shrubland
Species richness	16	16	17
<i>H</i>	2.66	2.68	2.71
<i>D</i>	0.92	0.92	0.92
<i>J</i>	0.96	0.96	0.95

## 4 Discussion

Our analysis of culturable soil fungi was small-scale and intended simply to detect the dominant groups for comparison among different sites by using rDNA ITS techniques. Four types of medium and a single set of growth conditions were used, and it is well known that isolation media can dramatically affect the overall diversity of species recovered (Frankland et al., 1990; Gams, 1992; Tabacchioni et al., 2000; Hirsch et al., 2010). However, there are a number of ecologically important fungal groups which are difficult to isolate, even with a more extensive culturing strategy. *Basidiomycetes* are notoriously difficult to isolate from soil (Frankland et al., 1990), but specialized culturing techniques, such as the modified soil washing procedure described, can be successfully applied, though such approaches are time consuming, require special identification skills, and still may not recover all taxa present (Tebbe and Vahjen, 1993; Thorn et al., 1996; Li et al., 2009). The isolation numbers and types of culturable soil fungi were influenced by many factors. The culturing conditions and the culture medium were dominant influence factors in the culture of the fungi isolated from the soil at an alpine site. In this study, a total of 35 strains out of 96 isolates were cultured and subcultured successfully. Although the culture media was carefully chosen to be as non-selective as possible, some species were not isolated probably because the culturing conditions and the culture medium were not appropriate for its growth. To obtain much more diversity data of the culturable soil fungi, the culturing conditions and medium as well as other factors should be optimized.

The most common representatives of fungi were collected in the three alpine grasslands of the Eastern Qilian Mountains. Ninety-six specimen's morphotypes were investigated. Molecular identification was carried out using sequence analysis of the rDNA ITS region. A total of 35 rDNA ITS sequences were compared by phylogenetic analyses using neighbor-joining and maximum parsimony. Traditionally, isolate identification is supported by morphology. From our study in assigning genera based only on the sequence similarity comparison, cluster analysis of the ITS sequences of the rDNA can agree well with initial mycelia sterilia morphotype groupings. Strains within each morphotype clearly grouped together as taxonomic units, whereas these different morphotypes being distinct taxa is unequivocal. In any case, the finding of a significant

number of cases in which the identification suggested by the molecular data are misleading compared to the morphological data, reveals the risk of giving absolute confidence to sequence data for identification purposes, at least for these fungal groups.

Molecular identification of fungi to a species level has generally utilized the variable ITS regions of the rDNA. However, although PCR primers exist with enhanced specificity for the ITS regions of *Basidiomycetes* (Gardes and Bruns, 1993) and *Ascomycetes* (Larena et al., 1999; Xu et al., 2012), they are generally unsuitable for direct amplification of fungal sequences from environmental samples for techniques such as ARDRA because they also amplify the ITS region from some plant material (Gardes and Bruns, 1993; Mello et al., 2011). In addition, the variability of the ITS region makes it unsuitable for broad-scale comparisons of fungal groups (Kuninaga et al., 1997; Hibbett et al., 2011), since sequence alignment becomes difficult (Anderson et al., 2003). Molecular techniques such as amplified rDNA sequencing, amplified 18S rDNA restriction analysis (ARDRA), and temperature and denaturing gradient gel electrophoresis (TGGE/DGGE) can be used to provide a culture-independent picture of soil microbial communities. Such techniques are now widely used in the study of soil bacterial diversity, but there have been relatively few attempts to assess their suitability to study fungal diversity in the field (Kowalchuk et al., 1997; van Elsas et al., 2000; Anderson et al., 2003; Unterseher et al., 2011) and apart from one study that only provided qualitative information. This study has shown that rDNA ITS based techniques can be used effectively as an initial screen to detect differences between soil fungal communities. However, rDNA ITS is not variable enough to distinguish among closely related species in all cases, and more variable genes or more variable parts of the rDNA are needed to completely describe diversity. Although the relatively small numbers of reference sequences currently available makes detection and identification of unknown clones problematic at present, this situation will improve as greater numbers of ITS fungal sequences become available.

This work has provided a more complete knowledge of soil fungi in alpine shrublands of the Eastern Qilian Mountains. New evidence for the possible indigenous nature of certain fungal species has been presented. A total of 22 taxa have also been identified that have 98% identity on best BLAST matches, indicating that their phylogenetic

relationships are clear and these species possibly belong to those previously reported. The results of this research also emphasize the importance of using molecular methods of detection in addition to traditional culturing methods in surveys of diversity to obtain a more precise analysis of the fungi present. Relative abundance of the fungi morphotypes within the soil samples was estimated. In this study, the results show that environmental factors had obvious effects on fungi diversity, especially the type of the shrub and the physical-chemical properties of soils. The vegetation composition had also a positive correlation with fungi diversity. Clearly these factors will influence the composition and abundance of fungal community members, although it is not possible to single out any one factor as a dominant one from the basic status of the sampling sites. It is often perceived that high plant diversity should promote a richly diverse fungal community due to the formation of intimate relationships between plant species and soil fungi. The composition of fungal species present in a shrub-grassland soil may have important implications for plant biodiversity and ecosystem variability, and ecosystem stability may be particularly affected.

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